A New Method of Bipolymerase Sequencing Prevents "Stop-Bands"

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Abstract

This article presents a relatively quick and cost-effective DNA sequencing method that prevents the formation of stop-bands. This method uses a combination of Taq and Sequenase that allows sequencing at both low and high temperatures. The ability to sequence at a high temperature appears to be the fundamental component in preventing stop-band formation in G+C rich regions.

Index Entries: Artifactual; DNA: polymerase; Taq; Sequenase.

Artifactual banding patterns, or stop-bands, are a common problem when sequencing DNA using the Sanger (dideoxynucleoside chain termination) method (1). Radioactive labeling at the same position in all four lanes occurs frequently in G+C rich areas and is presumably the result of the stalling or pausing of the polymerase enzyme in regions of extreme secondary structure (2). Stop-bands are problematic because they prevent the elucidation of the correct nucleotide sequence. Several methods have been proposed for eliminating these stop-bands (2–5). Unfortunately these methods have met with only moderate and intermittent success in preventing stop-band formation.

Here I report a relatively quick and cost-effective method for eliminating stop-bands (Fig. 1). This method involves the combination of two polymerase enzymes, Taq and Sequenase Version 2.0 (United States Biochemical, Cleveland, OH), that, unlike other bipolymerase sequencing methods (3), provides the benefit of being able to sequence both at low and high temperatures. The ability to sequence at a higher temperature appears to be the key determinant in eliminating the secondary structures that cause stop-bands. I have found that bipolymerase sequencing not only prevents the formation of hard stop-bands (Fig. 1, closed arrow), but also helps reduce ghost or background banding patterns (Fig. 1, open arrow), making the autoradiographs easier to read. This method has proven to be effective and consistent on diverse taxa (lizards and bacteriophage) and produces qualitatively better results than sequential sequencing techniques (4).

Double-stranded plasmid DNA was prepared for sequencing by bringing up 2–10 μg of DNA in 19 μL of ddH₂O and 1 μL of 5M NaOH and incubating at 65°C for 5 min. Two microliters of 7M CH₃COONH₄, 9 μL of 1M CH₃COONa, and 14 μL of ddH₂O were added and mixed thoroughly. One hundred thirty microliters of 95% EtOH were added and this mixture was incubated at −20°C for 15 min. Samples were spun at maximum speed in a microfuge for 15 min after which time the supernatant was removed. One hundred thirty microliters of 70% EtOH were added and spun in a microfuge for another 15 min. The supernatant was discarded and the microfuge tube with the pelletted DNA was placed in a 37°C incubator for 5 min to evaporate off any remaining EtOH.

The dried samples were resuspended in 7 μL of ddH₂O, 1 μL primer, and 2 μL 5X Sequenase Buffer, incubated for 2 min at 65°C, allowed to cool slowly to room temperature and then placed...
Table 1
Protocol for the Elimination of Stop-Bands Using Bipolymerase Sequencing

1. Follow the Sequenase Version 2.0 protocol for the annealing reaction.
2. Follow the Sequenase Version 2.0 protocol for the labeling reaction, except add 0.50 µL Taq.
3. After the labeling reaction is complete, add 3.5 µL of the labeling reaction to each of the termination tubes with 2.5 µL of the appropriate dideoxynucleoside (ddNTP) termination mixture. Incubate at 37°C for 5 min.
4. Place the four termination tubes in a 95°C bath for 1.5 min, remove and spin briefly, quickly add an additional 2.0 µL of the appropriate dideoxy mix to each tube, and then incubate at 73°C for 45 min. Add 4.0 µL of stop solution.

1.5 min, quickly spun in a microfuge, and placed in a 73°C water bath. Immediately after being placed in the 73°C bath, 2.0 µL of the appropriate dideoxynucleoside mix were again added to the termination reaction tubes. The termination tubes were then incubated at 73°C for 45 min after which time 4 µL of stop solution were added to each tube (Table 1).

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References